白頭翁素抑制人類正常黑色素細胞內酪氨酸酵素活性與酪氨酸相關酵素表現

Anemonin inhibits the tyrosinase activity and expression oftyrosinase-related proteins in human epidermal melanocytes

中文摘要

在黑色素生成步驟裡酪氨酸相關酵素共同參與其反應過程，包含酪氨酸酵素、酪氨酸相關酵素 1 和酪氨酸相關酵素 2。酪氨酸酵素是個含銅的單氧酶，在生成黑色素中進行羥基化與氧化反應此爲合成黑色素的速率決定步驟。

本實驗的 20 種純化合物由天然物分離出來，包含植物、黴菌、海洋細菌與海藻，拿來篩選對黑色素細胞內酪氨酸酵素的活性與抑制黑色素生成的效果。在實驗過程中，一開始化合物在 50 μM 的濃度時處理正常人類黑色素細胞 24 小時，得到結果爲 10 種化合物的細胞存活率>80%。此 10 種化合物接下來也以 50 μM 進行對酪氨酸酵素活性的探討。在此步實驗結果顯示，白頭翁素(Anemonin)對酪氨酸酵素的抑制活性能大於 50%。

由上述的篩選過程中發現白頭翁素(Anemonin)由厚葉鐵線蓮(Clematis crassifolia Bentham)萃取而來，具有抑制黑色素細胞內酪氨酸酵素的能力其 IC50 爲 43.5 μM，當細胞加藥處理 48 小時，酪氨酸酵素抑制率可高達 60%以上。在抑制黑色素生成方面，加藥 24 小時後對黑色素的生成並無明顯的影響，但持續加藥 48 小時後黑色素生成可將低約 70%左右。

因此在確定黑色素被抑制後進一步的討論白頭翁素對黑色素細胞體內機制的探討，在西方點墨法的結果酪氨酸酵素與相關酵素 2 的蛋白表現在 24 小時後都被明顯的抑制，但酪氨酸相關酵素 1 的蛋白表現在 48 小時後才有明顯的抑制。以細胞免疫染色法加以觀測細胞內此 3 種酵素，酪氨酸酵素與酪氨酸相關酵素 2 在細胞體內的表現是降低的。最後利用即時定量聚合酵素連鎖反應觀察 3 種酵素的基因表現，白頭翁素確實能減少酪氨酸及其相關蛋白的基因表現。

本實驗結果討論，白頭翁素在影響黑色素的生成原因可能是經由抑制酪氨酸及其相關酵素的基因表現，因此能調控此 3 種酵素的蛋白表現。白頭翁素對黑色素細胞在不產生細胞毒性之下能夠抑制黑色素的生成，但完整的作用機制尚未明確，希望在未來可以實際運用在化妝品美白的用途上，做爲新一代皮膚美白劑。

英文摘要

Melanin synthesis is a highly cooperative process carried out by tyrosinase family proteins, including tyrosinase, tyrosinase-related protein 1 (TRP1) and tyrosinase-related protein 2 (TRP2). Tyrosinase is a copper-containing

monooxygenase. Tyrosinase catalysis reaction including hydroxylation and oxidation steps which are the rate-limiting steps in melanin production.

In our study, 20 compounds isolated from natural products, including plants, fungus, ocean bacteria and seaweed, were evaluated their ability to inhibit cellular tyrosinase activity and reduce melanin synthesis in human epidermal melanocyte (HEMn).

Among the compounds, cell viabilities of 10 compounds were>80%, when cells were treated with 50 μM of different compounds for 24 hours. They were further evaluated the tyrosinase activity. Among these compounds, Anemonin isolated from Clematis crassifolia Bentham showed the most efficacious in inhibition of tyrosinase activity. When treated with Anemonin for 48 hours, the inhibition of tyrosinase activity was above 60%. Melanin synthesis was reduced to 70% when sustained administration for 48 hours. But it showed no variation in melanin synthesis when administration for 24 hours.

The cellular mechanism in melanocyte was further investigated. The protein expression of tyrosinase and TRP2 were significantly Inhibited in 24 hours but the protein expression of TRP1 were inhibited until 48 hours later. Decreased of these protein expression were further confirmed by immunocytochemistry. Anemonin suppressed the expression of tyrosinase and it's related proteins as demonstrated by quantitative real-time PCR (qRT-PCR).

In conclusion, Anemonin may down-regulated gene encoding of tyrosinase and it's related proteins (TRP2 and TRP2) and resulted in reducing melanin synthesis. Anemonin can inhibit melanin synthesis without cytotoxicity in human melanocyte although cellular mechanism was still unclear. Our results suggest that Anemonin can be used as whitening agent for therapeutic intervention of hyperpigmentation on cosmetics.